United States Environmental Protection Agency **Office of Research and Development**

National Exposure Research Laboratory
Research Abstract
Government Performance Results Act Goal: Clean Water

Significant Research Findings:

Infective Dose of *Cryptosporidium* in Immunocompromised Hosts

Scientific Problem and Policy Issues

Recent evidence regarding the safety of drinking water indicates that established and emerging pathogens continue to pose a public risk. Outbreaks of disease linked to drinking water have been described for *Cryptosporidium*, a microbe that causes gastroenteritis of varying severity and sometimes causes death. In 1993, a large waterborne outbreak in Milwaukee, Wisconsin, caused over 400,000 cases of gastroenteritis and resulted in over 100 deaths.

The U.S. EPA has set a goal of 0 cases of cryptosporidiosis due to waterborne transmission. In addition to being a waterborne disease, cryptosporidiosis can be transmitted by fecal oral contact, (e.g., in day care centers, through food, or through certain sexual practices). If fewer than 1 in 10,000 people in the U.S. become ill from waterborne cryptosporidiosis, it may be impossible to determine if these infections were due to water or through some other mode of transmission. It should be possible to calculate the maximum permissible number of *Cryptosporidium* infectious bodies (or oocysts) per unit of water that would ensure a case rate no greater than 1 in 10,000 persons per year. This calculation relies on knowing the infectious dose of *Cryptosporidium* oocysts, or the number of oocysts required to make a person sick. Studies in healthy, immunocompetent human volunteers have shown that the dose that causes infection in 50 percent of the subjects ranges between 10 and 1000 oocysts, depending upon the *Cryptosporidium* strain.

It is thought that natural, non-specific immunity is the reason why the infectious dose of *Cryptosporidium* is greater than 1 oocyst per individual. Because of this, it is possible that people with compromised immune systems are susceptible to infections at doses lower than immunocompetent individuals. There is no FDA approved treatment for this disease; however, immunocompetent individuals generally recover without treatment within several weeks. People who are immunocompromised may be unable to suppress the disease with their immune system, and in these people, the disease may last longer,

	or may even be fatal. Consequently, it is not possible to study the infective dose in immunocompromised human volunteers. Because the number of immunocompromised people is increasing in the United States, it is important to determine if there is a difference between the infective dose in the immunocompetent and immunocompromised host. This information is needed for risk assessment modeling and, ultimately, for development of water treatment industry regulations.
Research Approach	Cryptosporidiosis could not be studied in immunocompromised humans because of the risk of adverse outcomes for these people. There was not an accessible animal model for *Cryptosporidium parvum* (the human infective species) in which fully immunocompetent individuals could be infected, as was seen in the human population. A related parasite, *Cryptosporidium muris,* infects immunocompetent mice, and this model was selected for its similarity to the human / parasite relationship. The experiment was designed to determine if there was a difference between the infectious dose in mice that were immunocompetent versus those that were immunocompromised. Standard operating procedures for handling these animals were obtained or developed and the experimental protocols were certified. Flow cytometry was selected as the most efficient and accurate method of counting the oocysts needed to dose the mice. The initial studies determined the dose that causes infection in 50 percent of the mice. The long-acting steroid methylprednisolone acetate injected intramuscularly was used to cause immunosuppression in the mice, since preliminary experiments showed that it produced a more controlled chemical immunosuppression of mice than another common technique, dexamethasone administered to mice in drinking water.
Results and Implications	White blood cell (leucocyte) counts in immunosuppressed C57 Bl/6 mice indicated that both the total white cell count and the CD4 and CD8 lymphocyte counts dropped substantially by 24 hours post methylprednisone injection. Lymphocytes expressing the CD4 antigen are considered to represent the helper T cell population which orchestrates both the antibody mediated and cell mediated immune responses. Lymphocytes expressing the CD8 antigen are thought to represent the specific cytotoxic T cell population that respond to and kill cells that are infected with intracellular pathogens. The total white cell count and the CD4 and CD8 lymphocytes counts did not recover significantly after two weeks, even though no remains of the drug could be observed at the injection site upon necropsy. This data indicates that methylprednisolone acetate is a suitable drug to chemically immunosuppress mice.

A statistical logic model was used to estimate the association between doses and infection rates between immunocompromised and immunocompetent animals. The infectious dose for 50% (ID_{50}) of the immunocompetent mice was 61 oocysts, and the calculated ID_{50} for the immunosuppressed mice was 102 oocysts. Statistically there is no meaningful difference in the dose-response between the immunocompetent and immunosuppressed mice ($P = 0.73$). The low p-value and counter-intuitive results are probably due to the low number of mice that have been tested in this dosage range. If there is a true difference between the infectious doses required for immunosuppressed versus immunocompetent mice, substantially more trials will be required in order to distinguish this difference from the experimental error.

Research Collaboration and Publications

This research was a collaborative effort between the National Exposure Research Laboratory and the National Health and Environmental Effects Research Laboratory.

Bennett J.W., M.R. Gauci, S. Le Moenic, F.W. Schaefer III, and H.D. Lindquist. 1999. A comparison of enumeration techniques for *Cryptosporidium parvum* oocysts. *Journal of Parasitology* 85:1165-1168.

Miller, T.A. and F.W. Schaefer, III. 2001. Use of the long acting steroid methylpredisolone acetate for the prolongation of *Cryptosporidium* in mice. VII International Workshop on Opportunistic Protists, June 13-16, Cincinnati, Ohio.

Future Research

Research planned for the future calls for additional replicate determinations of the infective dose in methylprednisolone acetate immunosuppressed mice. Infective dose of *Cryptosporidium* will also be established between genetically (rather than chemically) immunocompromised and immunocompetent mice. Chemically immunosuppressing mice may have a different effect on the non-specific immunity of these mice than does genetic immune compromization.

Contacts for Additional Information

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